

Amendments to the Specification

Please replace the title on page 1, line 3 with the following title.

METHODS [METHOD] OF SCREENING FOR USEFUL PROTEINS

[PROTEIN]

Please replace the paragraph beginning at page 16, line 14 with the following paragraph.

The above-described proteins can be immobilized on the surface of a solid phase by known methods, which include, for example, methods utilizing tannic acid, formalin, glutaraldehyde, pyruvic aldehyde, bisdiazotized benzidine [bendizone], toluene-2,4-di-isocyanate, carboxyl group, hydroxyl group, or amino group (see P. M. Abdella, P. K. Smith, G.P. Royer, A New Cleavable Reagent for Cross-Linking and Reversible Immobilization of Proteins, Biochem. Biophys. Res. Commun., 87, 734 (1979); etc.).

Please replace the paragraph beginning at page 24, line 26 with the following paragraph.

Using the DNA library as a template and DNA primers
(CATTACATTTACATTCTACAAC TACAAGCCACCATG (SEQ ID NO: 6); and

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TTTCCCCGCCCCCGTCCT (SEQ ID NO: 7)), PCR was carried out (under the condition of: thermal denaturation at 95°C for 2 min, followed by 30 cycles of thermal denaturation at 95°C for 30 sec, annealing at 69°C for 15 sec, and extension at 72°C for 45 sec). The amplification product was purified by ethanol precipitation. Then, a DNA comprising the promoter sequence and non-coding region sequence of T7 (hereinafter abbreviated as T7Ω)

(GATCCCGCGAAATTAAATACGACTCACTATAGGGGAAGTATTTTACAACAAT TACCAACAAACAACAAACAAACAACAAACATTACATTACATTCTACAAC ACTAAGGCCACCATG (SEQ ID NO: 8)) was linked thereto by PCR (under the condition of: thermal denaturation at 95°C for 2 min, followed by 30 cycles of thermal denaturation at 95°C for 30 sec, annealing at 60°C for 15 sec, and extension at 72°C for 50 sec) without any primers. The resulting products were purified by phenol extraction and ethanol precipitation. Eventually, a template DNA was synthesized comprising, on the 5'-side, T7, Cap, omega sequence, and Kozak sequence and, on the 3'-side, a tag sequence partially complementary to spacer DNA (5' - AGGACGGGGGGCGGGGAAA [5' - AGGACGGGGGGCGGGGAAA] (SEQ ID NO: 9); the region complementary to the spacer sequence is underlined) in addition to a x6 histidine tag.

Please replace the paragraph beginning at page 28, line 10 with the following paragraph.

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The DNA obtained in Step 13 [3] described above was returned to Step 3 described above for transcription, and then the cycle of Steps 4 to 13 was repeated three times.

Please replace the paragraph beginning at page 34, line 17 with the following paragraph.

Specifically, the two DNAs encoding the sequences as described above were synthesized by Fasmac Ltd, and 100 pmol [pM] each of the DNAs were dissolved in 20 mM Tris-HCl buffer. Hybridization was performed through annealing which was achieved by heating at 95°C for 5 min and then slowly cooling to room temperature in 20 min. The product was treated with restriction enzymes *Bam*HI and *Xho*I, and then reacted with pGEM-1λEcoR1/BAP (similarly treated with *Bam*HI and *Xho*I, and then purified) using Quick Ligation Kit (New England Biolab) for 1 hr. *E. coli* cells were transformed with the constructed plasmid to select clones that comprise a plasmid of interest according to the Amersham Bioscience's protocol for expression and detection of GST fusion proteins. The clones were sequenced for confirmation. Then, the *E. coli* cells were cultured according to a general protocol. After the *E. coli* cells were heated in SDS-comprising buffer for 5 min at 95°C, analysis using 12% SDS-PAGE was carried out. After confirmation via CBB staining, the expression of the GST fusion protein was confirmed by Western blotting using goat anti-GST antibody. Then, the protein was purified according to the protocol appended to Bulk GST Purification Module of

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Amersham Bioscience. After culturing the *E. coli* cells in 100 ml of culture medium, the culture fluid was centrifuged at 8,000 x g for 10 min. The collected bacterial pellet was suspended in the Starting buffer, and sonicated on ice. Insoluble material was removed by centrifugation at 12,000 x g at 4°C for 10 min. Then, the resulting precipitate was suspended in the Starting buffer. The supernatant and precipitate were subjected to SDS-PAGE to confirm the presence of the protein of interest. Then, glutathione was removed from the buffer using Hitrap Desalting column according to the protocol of GSTrap FF of Amersham Bioscience. In addition to the protein in which the GST tag was removed by cleavage through the procedure as above, a protein that had been purified without the cleavage of the GST tag was prepared.